# Extracellular Signal-Regulated Kinase 2 Signaling in the Hippocampal Dentate Gyrus Mediates the Antidepressant Effects of Testosterone

Nicole Carrier and Mohamed Kabbaj

**Background:** Human and animal studies suggest that testosterone may have antidepressant effects. In this study, we sought to investigate the molecular mechanisms underlying the antidepressant effects of testosterone within the hippocampus, an area that is fundamental in the etiology of depression.

**Methods:** The effects of testosterone replacements in gonadectomized adult male rats were investigated using the sucrose preference and forced swim tests. We explored possible effects of testosterone on hippocampal neurogenesis and gene expression of stress-related molecules. Through the use of viral vectors, we pursued the antidepressant molecular mechanism(s) of testosterone in mediating anhedonia and manipulated extracellular signal-regulated kinase 2 (ERK2) expression in the dentate gyrus in gonadectomized rats with testosterone replacements.

**Results:** Testosterone had antidepressant effects, likely mediated by aromatization to estrogen metabolites, in the sucrose preference and forced swim tests despite having no effects on hippocampal cell proliferation or survival. We found a testosterone-dependent regulation of hippocampal ERK2 expression. Functionally, reducing ERK2 activity within the dentate gyrus induced anhedonia in gonadectomized rats receiving testosterone supplementation, whereas the overexpression of ERK2 rescued this behavior in gonadectomized rats.

**Conclusions:** These results implicate a role for ERK2 signaling within the dentate gyrus area of the hippocampus as a key mediator of the antidepressant effects of testosterone.

**Key Words:** 17β-estradiol, depression, ERK2, hippocampus, neurogenesis, testosterone

Affective disorders are twice as likely to occur in women as in men (1–4), implicating a critical role for gonadal hormones in their etiology. In particular, testosterone has mood-enhancing properties and antidepressant effects in men (5). In fact, increased incidence of hypogonadism occurs in men with major depressive disorder (MDD) (6,7), and testosterone replacement effectively improves mood (7–9). In rodents, testosterone has antidepressant effects in aged male mice (10) and protective effects against the development of depression-like behaviors in rats (11). These studies suggest a modulatory role for testosterone in the regulation of depressive disorders; however, the molecular mechanism(s) and brain site(s) of its actions are not well characterized.

Alterations of neurotrophic factors including brain-derived neurotrophic factor (BDNF) in limbic regions, such as the hippocampus, are associated with the treatment and/or onset of depression (12). Because increased BDNF expression and enhancement of neurogenesis in the dentate gyrus (DG) occur following treatment with antidepressants (13–18), it is possible that the antidepressant effects of testosterone are mediated through an increase in hippocampal BDNF expression and neurogenesis.

In addition to neurogenic changes, antidepressant treatments are associated with neuroendocrine regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Indeed, major depression is associated with dysregulation of the HPA axis, possibly reflecting decreased glucocorticoid receptor (GR) activity (19). In fact, GR function imbalance might be a contributing factor in depression. Moreover, antidepressant treatments increase GR expression in the hippocampus (20), suggesting a key role for GR in the development and treatment of depression (for review, see Anacker *et al.* [21]).

Testosterone may interact directly with androgen receptors or through aromatization to estrogen metabolites in the brain to stimulate the mitogen-activated protein kinase (MAPK) pathway (22), a fundamental signaling pathway and critical regulator of emotional responses. Chronic stress is associated with decreased protein expression of extracellular signal-regulated kinase 2 (ERK2) in the hippocampus. Both stress-induced depressive-like behaviors and ERK2 expression were reversed by chronic treatment with fluoxetine (23). Furthermore, chronic administration of lithium or valproate, mood stabilizers used in the treatment of manic depression, stimulates the MAPK pathway in the rat hippocampus (24). Interestingly, depressive-like symptoms were negatively correlated with ERK2 activation in the rat hippocampus (25). These studies implicate ERK activity within the hippocampus as a potential molecular target in the treatment of depression.

In this work, we investigated the role of testosterone and its metabolites in mediating depressive-like behaviors and explored possible underlying molecular mechanisms. In gonadectomized rats, we examined effects of testosterone on hippocampal cell proliferation and survival and changes in gene expression of stress-related molecules including BDNF, GR, and ERK2. Using viral vectors, we manipulated ERK2 activity in the DG and provided evidence for the role of testosterone-dependent ERK2 activity in mediating depressive-like behavior.

## Methods and Materials

### Experimental Design

**Experiment 1: The Effects of Testosterone and Its Metabolites on Depressive-Like Behaviors.** Rats were sham-operated or gonadectomized and received placebo, low-dose, or high-dose tes-

From the Department of Biomedical Sciences, Program in Neurosciences, College of Medicine, Florida State University.

Address correspondence to Mohamed Kabbaj, Ph.D., Associate Professor, Biomedical Sciences & Neurosciences, College of Medicine, 1115 West Call Street, Tallahassee, FL 32306; E-mail: Mohamed.Kabbaj@med.fsu.edu.

Received Aug 23, 2011; revised Nov 7, 2011; accepted Nov 30, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.11.028

**Table 1.** Volume (Mean ± SEM) of the Dentate Gyri

| Experiment | Treatment | Dentate Volume ($mm^3$) |
|---|---|---|
| Cell Proliferation | Gnx +Placebo | 9.033 ± .377 |
| | Gnx + Testosterone | 9.145 ± .322 |
| | Sham + Saline | 8.273 ± .237 |
| | Sham + IMI | 8.502 ± .261 |
| Cell Survival | Gnx + Placebo | 8.098 ± .173 |
| | Gnx + Testosterone | 8.916 ± .351 |

There were no significant differences among groups.
Gnx, gonadectomized; IMI, imipramine.

tosterone pellet implants. Twenty-one days later, all rats underwent the sucrose preference test (SPT) followed 1 week later by the forced swim test (FST). Rats were sacrificed 7 days later under basal, nonstressful conditions, hippocampi were collected, and the effects of gonadectomy and testosterone replacements on the expression of stress-related molecules (BDNF, GR, ERK2) was examined. A separate group of rats were gonadectomized and received placebo, β-estradiol 3-benzoate, or 5α-dihydrotestosterone (DHT) supplementation. Their behavior was analyzed 2 weeks later in the FST.

**Experiment 2: The Effects of Testosterone on Neurogenesis.** Rats were gonadectomized and received placebo ($n = 12$) or high-dose ($n = 12$) testosterone pellets. To examine cell proliferation, rats were injected with bromo-2′-deoxyuridine (BrdU) 20 days after gonadectomy and sacrificed 24 hours later. To examine cell survival, rats were injected with BrdU on three occasions, once every 24 hours, beginning on the day of gonadectomy and sacrificed 21 days later. Saline or imipramine contained in mini osmotic pumps was administered to control rats to examine the effects on cell proliferation (protocol adapted from Spritzer and Galea) (26).

**Experiment 3: The Role of ERK2 in the Antidepressant Effects of Testosterone.** Sham-operated and gonadectomized rats receiving placebo or low-dose testosterone pellet supplementation were injected with herpes simplex virus (HSV) viral vectors containing green fluorescent protein (GFP), dominant negative ERK2-GFP (dnERK2), or the ERK2-overexpressing GFP form (wtERK2) into the DG. All rats underwent the SPT for 7 days beginning 24 hours after viral injections. Rats were sacrificed on the eighth day and brains were collected for injection placement verification.

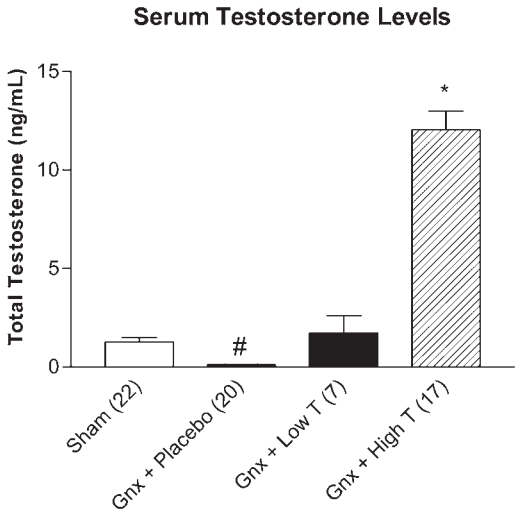


**Figure 1.** Serum testosterone levels (mean ± SEM) from sham gonadectomized ($n = 22$) and gonadectomized animals receiving placebo ($n = 20$), low-dose ($n = 7$), or high-dose testosterone supplementation ($n = 17$). $^{*}p < .0001$ compared with sham, gonadectomized + placebo, low-, and high-dose testosterone. $^{\#}p < .05$ compared with sham, gonadectomized + low- and high-dose testosterone treatment groups. Gnx, gonadectomized.

## General Methods

**Animals.** Adult male Sprague-Dawley rats weighing 250 to 270 g were purchased from Charles River (Wilmington, Massachusetts), pair-housed in 43 × 21.5 × 25.5 cm plastic cages, and kept on a 12 hour–12 hour light– dark cycle (lights on at 7:00 AM). Food and water was available ad libitum except during testing. All behavioral experiments, except the SPT, were conducted during the first 4 hours of the light phase of the light– dark cycle and were in accordance with the National Institutes of Health *Guide for Care and Use of Laboratory Animals* and approved by the Institutional Animal Care and Use Committee of Florida State University.

**Surgery.** Rats were anesthetized with a ketamine (70 mg/kg)/xylazine (10 mg/kg) mixture (intraperitoneal [IP] injection). Bupivacaine (.25% solution; .4 mL/kg) was applied topically as analgesic, and the nonsteroidal anti-inflammatory drug meloxicam (1.0 mg/mL) was injected subcutaneously.

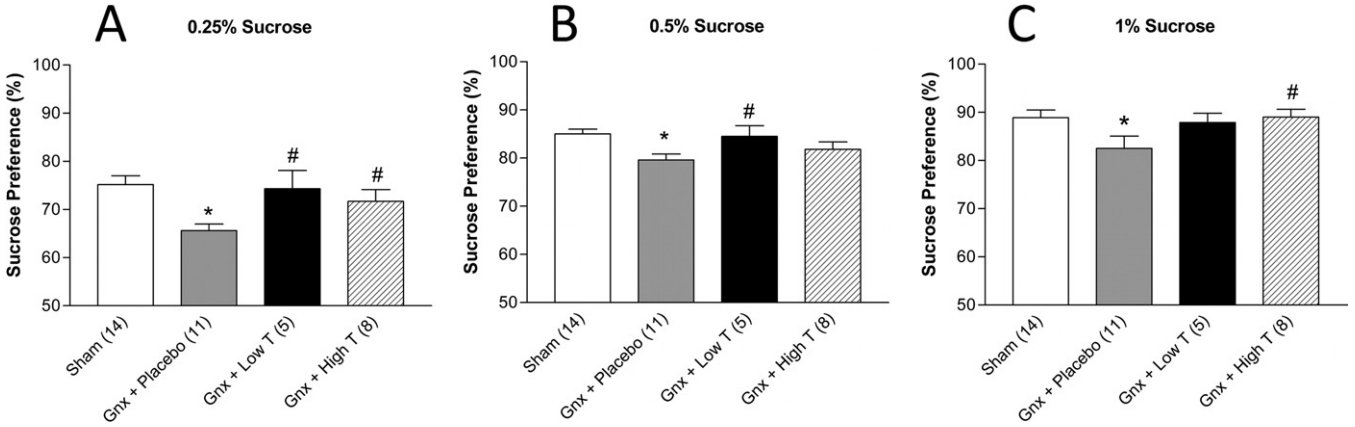


**Figure 2.** Sucrose preference. Gnx + placebo rats exhibit decreased sucrose preference at **(A)** .25%, **(B)** .5%, and **(C)** 1% sucrose concentrations, compared with sham Gnx and Gnx animals receiving low- and high-dose testosterone (T) supplementation ($n = 5$–14 per group). $^{*}p < .05$ compared with sham. $^{\#}p < .05$ compared with placebo. Gnx, gonadectomized.